

Appraisal of antimicrobial properties of *Onosma bracteatum* Wall. fruit extracts against respiratory tract pathogens

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ABSTRACT

The fruit extracts of *Onosma bracteatum* was extracted with petroleum ether, acetone, methanol, and aqueous solvents and screened for their antimicrobial activity against five bacteria and one fungus causing respiratory tract infections. All extracts showed inhibitory activity against selected pathogens. The crude extracts showed varying levels of bactericidal activity. The methanol extract showed maximum activity ranged between 12.6 ± 0.28 and 20.6 ± 0.28 mm at 200 mg/ml. The antifungal activity noted highest with 24.74% inhibition by methanol extract at 250 mg/ml. *O. bracteatum* can be a suitable source for new antibacterial drug development to cure respiratory diseases.

KEY WORDS: Antimicrobial, crude extracts, *Onosma bracteatum*, respiratory tract pathogens

INTRODUCTION

The respiratory tract is a complex organ of the human body in itself. It is directly exposed to the outer environment by continuous air inhalation. Several microorganisms are commonly associated with aerosols entered into the respiratory system with air and may be a major factor for infections. The upper respiratory tract includes the infections, i.e., sinusitis, rhinitis, tonsillitis, pharyngitis, laryngitis and common cold, and lower respiratory tract with bronchitis and pneumonia. The most common pathogens associated with such infections are *Staphylococcus aureus*, *Staphylococcus epidermidis*, α - and γ -Streptococci (Brook, 1988; Savolainen *et al.*, 1986), *Aspergillus fumigatus*, *Aspergillus niger* (Jahrsdoerfer *et al.*, 1979; Morgan *et al.*, 1984), *Streptococcus pneumoniae*, *Streptococcus pyogenes* (Dunne *et al.*, 2013), *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Legionella* (Ortqvist *et al.*, 2005), respectively.

Onosma bracteatum Wall. (Boraginaceae), is commonly known as Gaozaban, Gojihva in India. The genus *Onosma* includes about 150 species distributed worldwide. *O. bracteatum* is native to the Mediterranean and Western Asia. In India, it is found abundantly in North-western

Himalayas to Kashmir. It is a biennial herb. The stem is simple, covered with minute spines, arising from a cluster of radical leaves. *O. bracteatum* is a key ingredient in a number of Ayurvedic and Unani formulations (Badruddeen *et al.*, 2012). It is prescribed in bronchial asthma and rheumatoid arthritis. Reports suggest its demulcent, diuretic, anti-inflammatory, antileprotic, spasmolytic, and tonic nature (Chopra *et al.*, 1986). It is used in the preparation of Joshandah generally imposed in the treatment of common cold, catarrh, cough and associated respiratory distress, and fever (Vohora, 1986).

O. bracteatum contains alkannin and shikonin, flavonoids, ferulic, and vanillic acids which represent its pharmacological values (Kumar *et al.*, 2013). The roots are used for coloring food stuffs, oils and dyeing wool and in medicinal preparations. *O. bracteatum* exhibited broad spectrum antibacterial activity against Gram-positive and negative bacteria causing gastrointestinal, respiratory, and dermatological disorders (Walter *et al.*, 2011). Hence, this study is a conscientious attempt to find out the antimicrobial potential of *O. bracteatum* fruit extracts against respiratory tract pathogens.

MATERIALS AND METHODS

Plant Material

O. bracteatum was supplied by Vijay Herbal Automation, Haridwar in which fruit part was collected separately. Fruits were washed properly in fresh water, dried under shade at room temperature. Finally, it was crushed to small pieces using pestle and mortar and powdered in an electric grinder.

Reagents and Chemicals

All solvents and chemicals were of high-performance liquid chromatography or analytical grade and purchased from Merck, India and media for microbiological purposes from HiMedia Laboratories Pvt. Ltd., India, respectively.

Preparation of Extract

The powdered fruits were extracted sequentially by immersing 200 g of material in 600 ml solvents including petroleum ether (PET), acetone (ACE), methanol (MeOH), and aqueous (H₂O) in a Soxhlet apparatus (Ahmad *et al.*, 1998) using the successive extraction method. The extracts were concentrated in a vacuum evaporator and stored at 4°C until use. The yield of PET extract was 7.3 g, ACE extract 9.0 g, MeOH extract 11.1 g, and H₂O extract 12.4 g, respectively. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200 mg/ml for the agar well diffusion method.

Microorganisms Used

The bacterial strains causing respiratory tract infections namely *H. influenzae* (MTCC 3826), *Pseudomonas aeruginosa* (MTCC 2474), *Staphylococcus aureus* (MTCC 1144), *S. pneumoniae* (MTCC 655), *S. pyogenes* (MTCC 442), and *A. niger* (MTCC 921) were procured from Institute of Microbial Technology, Chandigarh.

Antibacterial Testing

The agar well diffusion method was used to screen the antibacterial activity (Ahmad *et al.*, 1998). This method depends on the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing a solution of tested material. Muller Hinton agar (MHA) was used for antibacterial screening. 0.1 ml of diluted inoculum (10⁵ CFU/ml) of test microorganisms were mixed in MHB and poured in sterilized Petri dishes. A cork borer (6 mm diameter) is used to punch wells

in medium and filled with 45 µl of plant extracts of 200 mg/ml final concentration of extracts. DMSO was used as negative control. Efficacy of extracts against bacteria was compared with a broad-spectrum antibiotic erythromycin (positive control). Each extract was assayed in triplicate. Plates were incubated at 37°C for 24 h in BOD incubator. The antibacterial activity was interpreted from the size of diameter of the zone of inhibition measured in millimeter.

Antifungal Testing

The antifungal activity of different extracts and essential oil was determined by poisoned food technique (Grover and Moore, 1962; Nene and Thapilyal, 2002). 250 mg/ml concentration of different plant extracts were aseptically poured into Petri plates followed by addition of 19 ml of melted Sabouraud dextrose agar medium and swirled gently to achieve thorough mixing of the contents. 6 mm mycelial discs from the margins of 2-day-old culture of *A. niger* was punched aseptically with a sterile cork borer and then put in the center of agar plates. In the control set, no extract was used. Percentage inhibition of mycelial growth was evaluated by measuring the relative growth of fungus in treatment and control and calculated using the following formula.

$$I = (C - T) / C \times 100$$

Where, I is the percentage inhibition, C the mean growth rate of control, and T that of the treatment.

The efficacy of extracts was compared with erythromycin as the reference drug. The plates were incubated at 25°C for 48-72 h in BOD incubator. Each sample was assayed in triplicate, and the mean values were observed.

Phytochemical Screening

The phytochemical analysis of plant extracts, i.e., PET, ACE, MeOH, and H₂O were carried out using standard qualitative methods for identification of various classes of active phytochemicals (Evans, 1996; Scalbert, 1991).

Test for Alkaloids

- The test solution was acidified with acetic acid, and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloids
- The test solution gave brown precipitate with the Dragendorff's reagent. The presence of brown precipitate showed positive test while absence of precipitate was negative.

Test for Flavonoids

- On addition of conc. HCl in MeOH extract of material, a red appeared which indicated the presence of flavonoids
- An ethanolic solution of test material was added with a small piece of magnesium ribbon, followed by drop wise addition of conc. HCl and change in color noted. The color was changed orange to red showed positive flavonoids test.

Test for Cardiac Glycosides

Plant extract was filtered, and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with $\text{Ba}(\text{OH})_2$. The remaining extract contained the glycosides. The hydrolysis of solution was done with conc. H_2SO_4 and after hydrolysis the presence of sugars was determined with the help of Fehling's solution.

Test for Steroids/Terpenes

Hosse's reaction

The extract mixed with 3 ml chloroform and 2 ml conc. H_2SO_4 was poured from the side of the test tube, and color of the ring at the junction of two layers was noted. A red showed the presence of steroids.

Moleschott's reaction

The extract was mixed with 5 ml distilled water and 2 ml conc. H_2SO_4 poured from the side of test tube and color was noted. A red appeared which changed to violet indicated presence of steroids.

Test for Saponins

Extracts (0.5 mg) were boiled with water (10 ml) for 2 min in a test tube and cooled. The mixture was shaken vigorously and left for 2-3 min. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Tannins

The extract was added in 1% ferric chloride and observed the color. Bluish black appeared which disappeared on the

addition of dilute H_2SO_4 follow a yellow-brown precipitate indicates the presence of tannins.

RESULTS AND DISCUSSION

The results showed that *O. bracteatum* has moderate antibacterial activity against tested microorganisms [Table 1]. The MeOH extract was found most active followed by H_2O , ACE, and PET. It showed maximum activity against *S. pneumoniae* (20.6 ± 0.28 mm) and lowest against *S. pyogenes* (12.6 ± 0.28 mm). The ACE and H_2O extracts were most active against *S. pneumoniae*, *P. aeruginosa*, and *S. aureus*. In comparison with erythromycin, the plant extracts were found less active. There was no inhibition noted with the negative control (DMSO). The antifungal activity of crude extracts showed significant inhibition effects on the mycelial growth of *A. niger* at 250 mg/ml. The most inhibition was noted by MeOH extract (25.3 ± 0.57 mm) with 24.74% and H_2O extract (27.6 ± 0.76 mm) with 17.82%, respectively. The control mycelial growth diameter was 33.6 ± 0.57 mm. The potency of crude extracts were compared with reference drug (erythromycin) showed 63.45% inhibition [Table 3].

In a study, Walter *et al.* (2011) observed the antibacterial effect of MeOH extracts of *O. bracteatum* leaves against *S. aureus*, *Escherichia coli*, and *P. aeruginosa*. The ranking of antibacterial activity against bacteria was *S. aureus* > *P. aeruginosa* > *E. coli*. In our study, *O. bracteatum* MeOH extract was found most effective against *S. pneumoniae* followed by *P. aeruginosa* and *H. influenzae*. *O. bracteatum* has been reported as major constituent used in the preparation of joshanda (Azmi *et al.*, 2010; Abdullah *et al.*, 2014) and Ayurvedic syrup (Sheikh *et al.*, 2014).

The preliminary phytochemical investigation for *O. bracteatum* was showed the presence of alkaloids, glycosides, steroids, terpenes, saponins, and tannins in MeOH extract; alkaloids, steroids, and terpene in PET extract; alkaloids, flavonoids, glycosides,

Table 1: Zone of inhibition of *O. bracteatum* fruit extracts against respiratory bacterial strains

Microorganisms	*Diameters of the inhibition zone (mm)				DMSO	Reference drug (erythromycin)
	PET	ACE	MeOH	H_2O		
<i>H. influenzae</i>	8.3 ± 0.28	10.3 ± 0.76	13.6 ± 0.28	12.3 ± 0.28	-	21.3 ± 0.76
<i>P. aeruginosa</i>	8.0 ± 0.50	10.3 ± 0.28	14.3 ± 0.76	13.6 ± 0.28	-	16.6 ± 0.76
<i>S. aureus</i>	8.0 ± 0.50	11.3 ± 0.76	12.3 ± 0.28	13.0 ± 0.50	-	30.3 ± 0.76
<i>S. pneumoniae</i>	9.3 ± 0.76	12.3 ± 0.28	20.6 ± 0.28	13.6 ± 0.28	-	19.3 ± 0.57
<i>S. pyogenes</i>	-	10.0 ± 0.50	12.6 ± 0.28	13.0 ± 0.50	-	25.6 ± 0.28

*Values are mean \pm SE, Cork borer diameter: 6 mm, -: No inhibition, SE: Standard error, *H. influenzae*: *Haemophilus influenzae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *S. aureus*: *Staphylococcus aureus*, *S. pneumoniae*: *Streptococcus pneumoniae*, *S. pyogenes*: *Streptococcus pyogenes*, PET: Petroleum ether, ACE: Acetone, MeOH: Methanol, DMOS: Dimethyl sulfoxide, *O. bracteatum*: *Onosma bracteatum*

Table 2: Phytochemical screening of *O. bracteatum* fruit extracts

S. No.	Phytoconstituents	Solvents			
		PET	ACE	MeOH	H ₂ O
1.	Al kaloids	+	+	+	-
2.	Flavonoids	+	+	+	+
3.	Glycosides	-	-	+	-
4.	Steroids	-	+	+	+
5.	Terpenes	-	+	+	+
6.	Saponins	-	-	+	+
7.	Tannins	-	-	+	+

+: Present, -: Absent, PET: Petroleum ether, ACE: Acetone, MeOH: Methanol, *O. bracteatum*: *Onosma bracteatum*

Table 3: Effect of plant extracts and erythromycin on the mycelial growth of *A. niger* at 250 mg/ml

Fungicide	*Mycelial growth (mm)	Control	% inhibition
PET	31.6±0.28	33.6±0.57	5.94
ACE	32.6±0.76	33.6±0.57	2.97
MeOH	25.3±0.57	33.6±0.57	24.74
H ₂ O	27.6±0.76	33.6±0.57	17.82
Erythromycin	12.3±0.57	33.6±0.57	63.45

*Values are mean±SE, SE: Standard error, PET: Petroleum ether, ACE: Acetone, MeOH: Methanol, *A. niger*: *Aspergillus niger*

steroids, terpenes, and saponins in ACE extract; and alkaloids, flavonoids, glycosides, steroids, saponins, and tannins in H₂O extract [Table 2]. Literature survey showed very little phytochemical work on *Onosma* genus. Aliphatic ketone, lipids (esters of palmitic acid and its homologues) (Mellidis and Papageorgiou, 1987a), naphthazarins (Mellidis and Papageorgiou, 1987b), alkaloids (Mellidis and Papageorgiou, 1988), and naphthoquinones (Khajuria and Jain, 1993) had reported from the *Onosma* genus. 10 pyrrolizidines alkaloids were identified from *O. arenaria* by Gas-liquid chromatography (GLC)-mass spectrometry and GLC including 5,6-dihydro-7,9-dimethoxy -7h- pyrrolizine, 7-acetylretronecine, 9-(butyryl-2-ene) supinidine, 7-acetyl-9-(2-methylbutyryl) retronecine, 7-acetyl-9-(2,3-dimethylbutyryl) retronecine, 7-acetyl-9-(2-hydroxy-3-methylbutyryl) retronecine, 3'-acetylupinine, 7-acetyl-9-(2,3-dihydroxybutyryl) retronecine, 7-acetylcopsamine, and uplandicine (El-Shazly and Abdel-Ghani, 2003).

Due to the traditional medicinal uses, *Onosma* species have been studied with their bioactive compounds. According to a recent review by El-Shazly and Wink, (El-Shazly and Wink, 2014) on pyrrolizidine alkaloids, the common metabolites of *Onosma* genus and *Boraginaceae* family, had figured out its toxicity, mutagenic properties for herbivores and humans, antimicrobial activities against human pathogens including *E. coli*, *S. pneumoniae*, *Bacillus subtilis*, *Bacillus anthracis*, and *S. aureus*, sequestration in insects for defense and mating purposes, and antifedant properties.

CONCLUSIONS

The present study represented the significant inhibitory role of *O. bracteatum* fruit extracts against selected respiratory tract pathogens. The MeOH extract found more potent in comparison to other solvents. *O. bracteatum* can be used in the treatment of respiratory diseases caused by selected microorganisms. The synergistic effect between antibiotics and plant extracts leads to the new choice of treatment. This is necessary to investigate the toxicity level of extracts resulting from over dosage or from any phytochemical component present in plant material.

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